AMENDMENTS

Amendments to the Claims:

This listing replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (previously presented) A method for selecting a rationally designed siRNA, wherein said rationally designed siRNA comprises 19 – 25 nucleoside base pairs, said method comprising:
   (a) selecting a target gene, wherein the target gene comprises a target sequence;
   (b) applying at least one non-target specific criterion to at least one candidate siRNA sequence, wherein the at least one non-target specific criterion comprises the presence or absence of a particular nucleotide at at least one of sequence positions 1-19 of the siRNA, the at least one non-target specific criterion increases a likelihood that said candidate siRNA sequence is functional, and the at least one candidate siRNA sequence comprises an antisense strand that is at least substantially complementary to a stretch of nucleotides of the target sequence; and
   (c) selecting a rationally designed siRNA, wherein said rationally designed siRNA comprises a sequence that satisfies said at least one non-target specific criterion.

2. (currently amended) The method according to claim 1, wherein said at least one candidate siRNA sequence comprises at least two candidate siRNA sequences, **and said applying at least one non-target specific criterion of step** (b) of claim 1 ~~further~~ comprises applying at least one of Formulas I, II, and IV-IX to each of said at least two candidate siRNA sequences, wherein said at least one of Formulas I, II, and IV-IX each determine a relative functionally of said at least two candidate siRNA sequences and wherein Formulas I, II, and IV-IX are:

Formula I = $-(GC/3) + (AU_{15\text{-}19}) - (Tm_{20°C})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula II = $-(GC/3) - (AU_{15\text{-}19})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula IV = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*2 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula V = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula VI = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula VII = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*1 - (G_{13})*3 - (C_{19}) + (A_{19})*3 + (A_3)*3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;

wherein in Formulas I, II, and IV – VII when applied to each of said at least two candidate siRNA sequences:

$AU_{15\text{-}19}$ = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20°C}$ = 1 if the Tm is greater than 20°C, otherwise its value is 0;

A_3 = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A_{14} = 1 if A is the base at position 14 on the sense strand, otherwise its

value is 0;

A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

U_5 = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein in Formula VIII and Formula IX when applied to each of said at least two candidate siRNA sequences

A_1 = 1 if A is the base at position 1 of the sense strand, otherwise its value is 0;

A_2 = 1 if A is the base at position 2 of the sense strand, otherwise its value is 0;

A_3 = 1 if A is the base at position 3 of the sense strand, otherwise its value is 0;

A_4 = 1 if A is the base at position 4 of the sense strand, otherwise its value is 0;

A_5 = 1 if A is the base at position 5 of the sense strand, otherwise its value is 0;

A_6 = 1 if A is the base at position 6 of the sense strand, otherwise its value is 0;

A_7 = 1 if A is the base at position 7 of the sense strand, otherwise its value is 0;

A_{10} = 1 if A is the base at position 10 of the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 of the sense strand otherwise its value is 0;

A_{13} = 1 if A is the base at position 13 of the sense strand, otherwise its value is 0;

A_{19} = 1 if A is the base at position 19 of the sense strand, otherwise if another base is present, its value is 0;

C_3 = 1 if C is the base at position 3 of the sense strand, otherwise its value is 0;

C_4 = 1 if C is the base at position 4 of the sense strand, otherwise its value is 0;

C_5 = 1 if C is the base at position 5 of the sense strand, otherwise its value is 0;

C_6 = 1 if C is the base at position 6 of the sense strand, otherwise its value is 0;

C_7 = 1 if C is the base at position 7 of the sense strand, otherwise its value is 0;

C_9 = 1 if C is the base at position 9 of the sense strand, otherwise its value is 0;

C_{17} = 1 if C is the base at position 17 of the sense strand, otherwise its value is 0;

C_{18} = 1 if C is the base at position 18 of the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise if another base is present, its value is 0;

G_1 = 1 if G is the base at position 1 on the sense strand, otherwise its value is 0;

G_2 = 1 if G is the base at position 2 of the sense strand, otherwise its value is 0;

G_8 = 1 if G is the base at position 8 on the sense strand, otherwise its value is 0;

G_{10} = 1 if G is the base at position 10 on the sense strand, otherwise its value is 0;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

G_{19} = 1 if G is the base at position 19 of the sense strand, otherwise if another base is present, its value is 0;

U_1 = 1 if U is the base at position 1 on the sense strand, otherwise its value is 0;

U_2 = 1 if U is the base at position 2 on the sense strand, otherwise its value is 0;

U_3 = 1 if U is the base at position 3 on the sense strand, otherwise its value is 0;

U_4 = 1 if U is the base at position 4 on the sense strand, otherwise its value is 0;

U_7 = 1 if U is the base at position 7 on the sense strand, otherwise its value is 0;

U_9 = 1 if U is the base at position 9 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

U_{15} = 1 if U is the base at position 15 on the sense strand, otherwise its value is 0;

U_{16} = 1 if U is the base at position 16 on the sense strand, otherwise its value is 0;

U_{17} = 1 if U is the base at position 17 on the sense strand, otherwise its value is 0;

U_{18} = 1 if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand;

GC_{total} = the number of G and C bases in the sense strand;

Tm = 100 if the targeting site contains an inverted repeat longer then 4 base pairs, otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a row, and (c) of claim 1 further comprises comparing the relative functionalities of said at least two candidate siRNA sequences, wherein the relative functionalities have been determined from the same formula and selecting as the rationally designed siRNA, one of the at least two candidate siRNA sequences that has a higher relative functionality than another of the at least two candidate siRNA sequences.

3. (previously presented) A method of gene silencing comprising selecting said rationally designed siRNA according to claim 2 and introducing said rationally designed siRNA into a cell *in vitro*.

4. (previously presented) The method according to claim 3 wherein said introducing is by allowing passive uptake of the rationally designed siRNA.

5. (original) The method according to claim 3, wherein said introducing is through the use of a vector.

6. (previously presented) A method for developing an siRNA algorithm for selecting a rationally designed siRNA, said method comprising:

(a) selecting a set of siRNA, wherein the set of siRNA comprises a plurality of siRNA that are directed against sequences in a target gene;

(b) determining the ability of each siRNA of said plurality of siRNA to silence the target gene;

(c) correlating the presence or absence of at least one variable with said ability of each siRNA to silence said target gene, wherein said at least one variable is selected from the group consisting of total GC content, melting

temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and

(d) developing an algorithm for selecting siRNA that are capable of silencing a target gene, wherein said algorithm comprises said at least one variable, and when the algorithm is applied to a candidate siRNA sequence the algorithm is capable of generating a value that is dependent on the satisfaction of said at least one variable and different values that are generated for different candidate siRNA sequences indicate a relative likelihood of functionality.

7. (canceled)

8. (previously presented) A method of selecting hyperfunctional siRNA, said method comprising measuring the silencing ability of said rationally designed siRNA of claim 1, wherein said silencing ability is measured *in vitro* at a concentration of less than 1 nanomolar siRNA, and selecting said rationally designed siRNA if said rationally designed siRNA either induces greater than 95% silencing or induces at least functional levels of silencing for greater than 96 hours.

9.-18. (canceled)

19. (currently amended) A kit for silencing a target gene, wherein said kit comprises at least two siRNA, wherein said at least two siRNA comprise a first optimized siRNA and a second optimized siRNA, wherein said first optimized siRNA and said second optimized siRNA each comprise <u>**a**</u> sense strand sequence~~s~~ of 18 –25 bases <u>**and an antisense strand sequence of 18 – 25 bases, and said sense strand sequences**</u> ~~that~~ are selected according to at least one of the following formulas and have first and second highest values generated by said at least one of

the following formulas for candidate siRNA that are directed against said target gene:

Formula I = $-(GC/3) +(AU_{15\text{-}19}) -(Tm_{20°C})*3 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) -(U_5) -(A_{11})$;

Formula II = $-(GC/3) -(AU_{15\text{-}19})*3 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3)$;

Formula III = $-(GC/3) +(AU_{15\text{-}19}) -(Tm_{20°C})*3$;

Formula IV = $-(GC/2)+(AU_{15\text{-}19})/2-(Tm_{20°C})*2 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) -(U_5) -(A_{11})$;

Formula V = $-(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) + (U_{10})+(A_{14}) -(U_5) -(A_{11})$;

Formula VI = $-(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3)$;

Formula VII = $-(GC/2) +(AU_{15\text{-}19})/2 - (Tm_{20°C})*1 -(G_{13})*3 -(C_{19}) +(A_{19})*3 +(A_3)*3 +(U_{10})/2+(A_{14})/2 -(U_5)/2 -(A_{11})/2$;

wherein in Formulas I – VII when applied to candidate siRNA sequences:

$AU_{15\text{-}19}$ = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20°C}$ = 1 if the Tm is greater than 20°C, otherwise its value is 0;

A_3 = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A_{14} = 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

U_5 = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein in Formulas VIII and IX when applied to candidate siRNA sequences:

A_1 = 1 if A is the base at position 1 of the sense strand, otherwise its value is 0;

A_2 = 1 if A is the base at position 2 of the sense strand, otherwise its value is 0;

A_3 = 1 if A is the base at position 3 of the sense strand, otherwise its value is 0;

A_4 = 1 if A is the base at position 4 of the sense strand, otherwise its value is 0;

A_5 = 1 if A is the base at position 5 of the sense strand, otherwise its value is 0;

A_6 = 1 if A is the base at position 6 of the sense strand, otherwise its value is 0;

A_7 = 1 if A is the base at position 7 of the sense strand, otherwise its value is 0;

A_{10} = 1 if A is the base at position 10 of the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 of the sense strand, otherwise its value is 0;

A_{13} = 1 if A is the base at position 13 of the sense strand, otherwise its value is 0;

A_{19} = 1 if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

C_3 = 1 if C is the base at position 3 of the sense strand, otherwise its value is 0;

C_4 = 1 if C is the base at position 4 of the sense strand, otherwise its value is 0;

C_5 = 1 if C is the base at position 5 of the sense strand, otherwise its value is 0;

C_6 = 1 if C is the base at position 6 of the sense strand, otherwise its value is 0;

C_7 = 1 if C is the base at position 7 of the sense strand, otherwise its value is 0;

C_9 = 1 if C is the base at position 9 of the sense strand, otherwise its value is 0;

C_{17} = 1 if C is the base at position 17 of the sense strand, otherwise its value is 0;

C_{18} = 1 if C is the base at position 18 of the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

G_1 = 1 if G is the base at position 1 on the sense strand, otherwise its value is 0;

G_2 = 1 if G is the base at position 2 of the sense strand, otherwise its value is 0;

G_8 = 1 if G is the base at position 8 on the sense strand, otherwise its value is 0;

G_{10} = 1 if G is the base at position 10 on the sense strand, otherwise its value is 0;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

G_{19} = 1 if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

U_1 = 1 if U is the base at position 1 on the sense strand, otherwise its value is 0;

U_2 = 1 if U is the base at position 2 on the sense strand, otherwise its value is 0;

U_3 = 1 if U is the base at position 3 on the sense strand, otherwise its value is 0;

U_4 = 1 if U is the base at position 4 on the sense strand, otherwise its value is 0;

U_7 = 1 if U is the base at position 7 on the sense strand, otherwise its value is 0;

U_9 = 1 if U is the base at position 9 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

U_{15} = 1 if U is the base at position 15 on the sense strand, otherwise its value is 0;

U_{16} = 1 if U is the base at position 16 on the sense strand, otherwise its value is 0;

U_{17} = 1 if U is the base at position 17 on the sense strand, otherwise its value is 0;

U_{18} = 1 if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

Tm = 100 if the targeting site contains an inverted repeat longer then 4 base pairs, otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a row.

20. (previously presented) The method according to claim 1, wherein the particular nucleotide is A and the sequence position is position 19.

21. (previously presented) The method according to claim 1, wherein said at least one candidate siRNA sequence comprises at least two candidate siRNA sequences.

22. (previously presented) The method according to claim 21, wherein said at least two candidate siRNA sequences comprise at least three candidate siRNA sequences.

23. (previously presented) The method according to claim 22, wherein said at least three candidate siRNA sequences comprise at least four siRNA sequences.

24. (previously presented) The method according to claim 23, wherein said at least four candidate siRNA sequences comprise all potential siRNAs sequences for said target gene that are 19 base pairs in length.

25. (previously presented) The method according to claim 1, wherein said antisense strand of said rationally designed siRNA is 100% complementary to said target sequence.

26. (previously presented) The method according to claim 24, wherein said antisense strand of said rationally designed siRNA is 100% complementary to said target sequence.

27. (previously presented) The method according to claim 6, wherein said at least one variable comprises at least two variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least two variables.

28. (previously presented) The method according to claim 27, wherein said at least two variables comprise at least three variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least three variables.

29. (previously presented) The method according to claim 28, wherein said at least three variables comprise at least four variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least four variables.

30. (previously presented) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular

nucleotides at at least two of sequence positions 1 - 19 of the at least one candidate siRNA, wherein said particular nucleotides may be the same or different nucleotides.

31. (previously presented) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least three of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two of more of said particular nucleotides may be the same or different nucleotides.

32. (previously presented) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least four of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two or more of said particular nucleotides may be the same or different nucleotides.

33. (previously presented) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least five of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two or more of said particular nucleotides may be same or different nucleotides.

34. (previously presented) The method according to claim 33, wherein said antisense strand is 100% complementary to said target sequence.

35. (previously presented) The method according to claim 1, wherein said rationally designed siRNA has a functionality of at least 70%.

36. (previously presented) The method according to claim 35, wherein said rationally designed siRNA has a functionality of at least 80%.

37. (previously presented) The method according to claim 36, wherein said rationally designed siRNA has a functionality of at least 95%.